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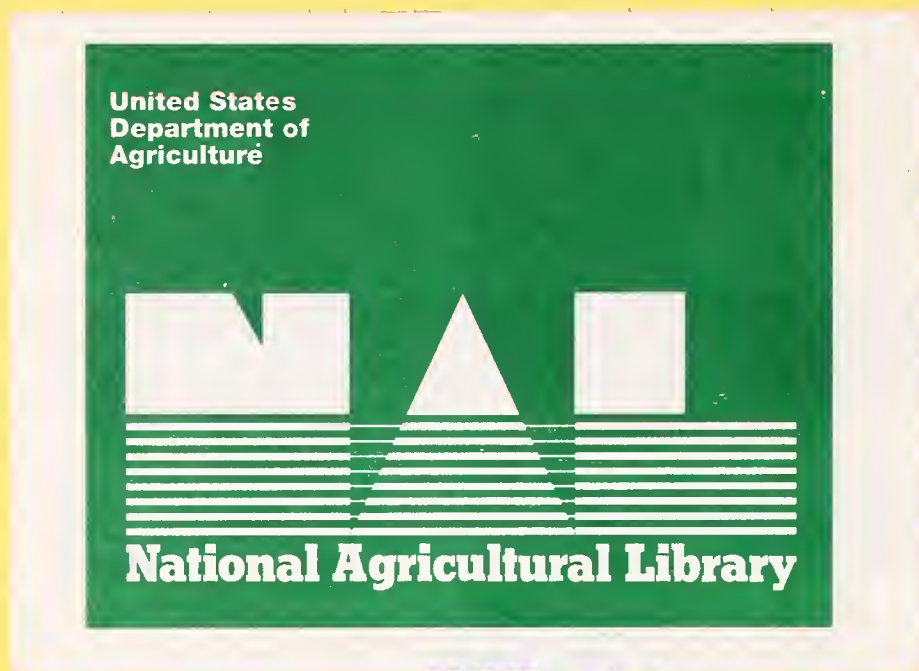
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## of CHESTNUT



This publication is one in a series on the genetics of important forest trees of North America being published by the Forest Service, U.S. Department of Agriculture, in cooperation with the Society of American Foresters. Development of this series is in accord with the resolutions of the World Consultation on Forest Genetics and Tree Improvement at Stockholm in 1963 and the Second World Consultation on Forest Tree Breeding at Washington, D.C., in 1969. The Committee on Forest Tree Improvement of the Society of American Foresters undertook the preparation of manuscripts for North American species.



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## CONTENTS

|                                                         | <i>Page</i> |
|---------------------------------------------------------|-------------|
| RESEARCH SUMMARY .....                                  | iii         |
| INTRODUCTION .....                                      | 1           |
| TAXONOMY.....                                           | 2           |
| SEXUAL REPRODUCTION.....                                | 2           |
| Flowering and Reproductive Development...               | 2           |
| Self- and Cross-Fertilization .....                     | 2           |
| Controlled Pollination .....                            | 3           |
| Seed Handling .....                                     | 3           |
| ASEXUAL REPRODUCTION .....                              | 4           |
| Rooting.....                                            | 4           |
| Grafting .....                                          | 4           |
| THE CHESTNUT BLIGHT FUNGUS AND<br>HOST RESISTANCE ..... | 5           |
| GENETICS AND BREEDING.....                              | 5           |
| Species Variability and Chromosome<br>Numbers.....      | 5           |
| Field Resistance of American Chestnut .....             | 6           |
| Interspecific Hybridization .....                       | 6           |
| Genetics of Resistance .....                            | 9           |
| Selected Blight Resistant Trees .....                   | 10          |
| IMPROVEMENT PROGRAMS .....                              | 11          |
| Screening for Disease Resistance.....                   | 11          |
| Systemic Fungicides .....                               | 11          |
| Research Needs .....                                    | 11          |
| LITERATURE CITED.....                                   | 12          |

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# GENETICS OF CHESTNUT

RESEARCH  
SUMMARY  
RP WO-17  
AUGUST 1974

## ABSTRACT

Jaynes, Richard A.

1972. Genetics of chestnut. USDA Forest Serv. Res. Pap. WO-17, -p.

The American chestnut (*Castanea dentata*) was destroyed as a commercial tree species by a blight fungus (*Endothia parasitica*). The efforts to develop blight-resistant chestnuts that have value for timber, wildlife food, orchard, and/or ornament are reviewed. Characteristics of chestnut including reproductive development, pollination techniques, asexual and sexual propagation, and blight resistance are reviewed as they apply to improvement of the species. Extensive interspecific hybridization work is discussed and suggestions on further work are offered.

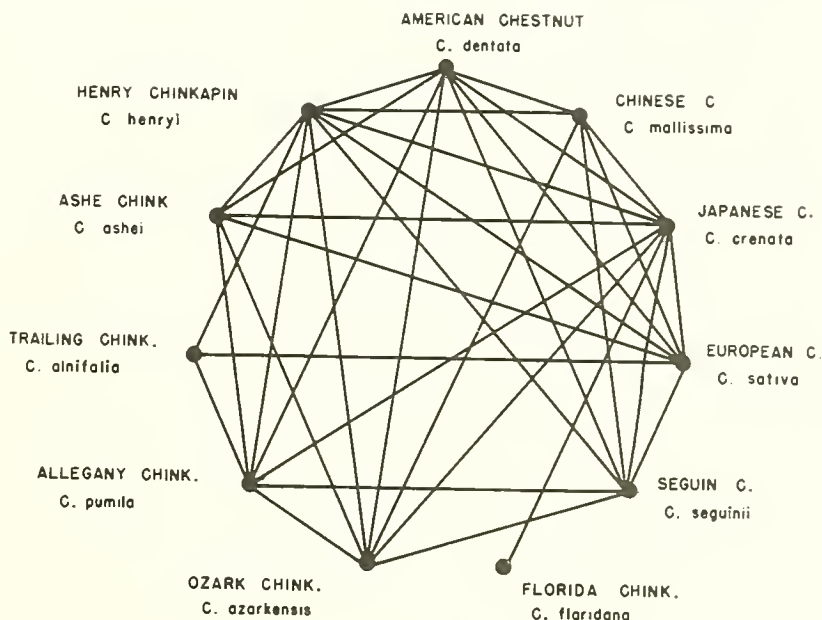
OXFORD: 165.4: 165.5: 443.3: 165.7: 181.5: 181.52. KEY WORDS: *Castanea*-spp., tree breeding, tree diseases, chestnut-blight, *Endothia-parasitica*, propagation, resistance, hybrids, pollination.

The American chestnut presently has no economic value as a timber tree, although it was once the most valuable hardwood in the deciduous forests of the eastern United States. A fungal parasite (*Endothia parasitica*) introduced from Asia destroyed the tree in the early 1900's. Oriental chestnut species are resistant to this disease but they generally have unsatisfactory forest-tree form. These blight-resistant species and some of their hybrids do have value as orchard trees, food producers for wildlife,

and ornamentals. Large numbers of Chinese chestnut seedlings are planted each year for home and game food production. Reproductive development, pollination techniques, and asexual and sexual propagation of chestnut trees are discussed in this paper.

The major objective in breeding chestnuts has been to obtain blight-resistant trees of upright form and vigor. Most  $F_1$  species hybrids have been attempted and there are few barriers to gene exchange among the species. This gives the breeder a large back-

Summary of successful controlled crosses between chestnut species as indicated by solid lines (Jaynes 1969b)



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ground of variability to draw upon. Blight resistance is not simply inherited but appears to be polygenic. The basis for blight resistance is unknown. Naturally occurring or irradiation-induced field resistance to the chestnut blight disease in the American chestnut has not been demonstrated.

Two areas where more information is vitally needed to assist the tree breeder are in screening for blight resistance and vegetative propagation. A rapid method to evaluate ultimate field resistance of seedlings is required to efficiently handle large numbers of plants. Preferably the technique should be based on a knowledge of the factors influencing host resistance. A commercially acceptable means to clonally propagate selections is needed to adequately test and disseminate improved cultivars.

Ultimately it may be possible through successive generations and the use of seed orchards to develop true-breeding, blight-resistant lines of chestnut. In the meantime, clonal propagation of selected clones will be necessary.

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# GENETICS OF CHESTNUT

by Richard A. Jaynes<sup>1</sup>

## INTRODUCTION

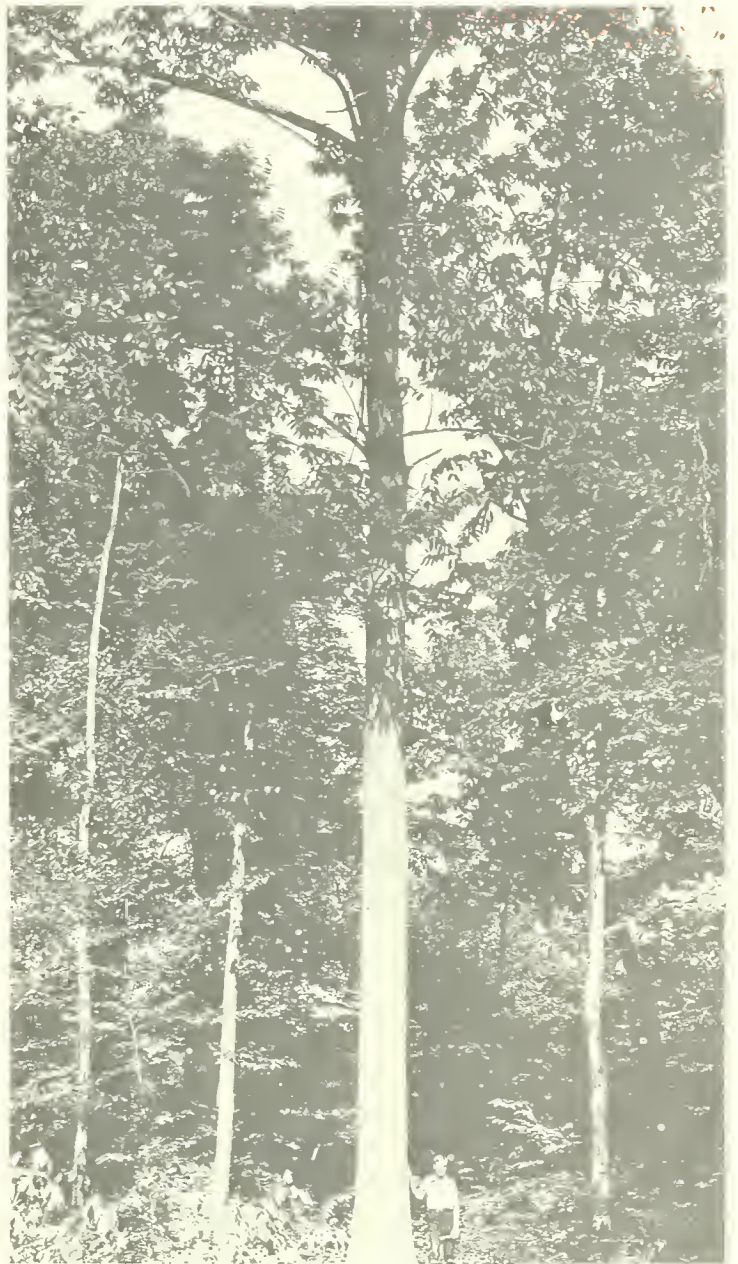
The American chestnut (*Castanea dentata* Borkh.) was once the most important forest tree in the deciduous forests of the eastern United States (fig. 1). The decay-resistant wood was useful in construction work and was almost the exclusive source of utility poles and railroad ties. Deer, squirrels, pheasants, turkeys, and many other animals depended on the nuts for food each fall. As a result of the introduction of a foreign pathogen in the late 1880's, the chestnut blight fungus (*Endothia parasitica* (Murr.) And.), the American chestnut was essentially destroyed within 40 years.

Field resistance of the American chestnut to this pathogen has not been demonstrated. However, blight resistance is characteristic of two Oriental chestnut species, the Chinese (*C. mollissima* Bl.) and the Japanese (*C. crenata* Sieb. and Zucc.). Forest geneticists and breeders have attempted by hybridization among these species to develop disease-resistant forest trees for the eastern United States. Most of our present knowledge of the genetics of chestnut stems directly from these efforts.

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Figure 1. An American chestnut in 1905 before the chestnut blight. The diameter at breast high was 27 inches. The tree was 83 feet tall and yielded 662 board feet of lumber.



## TAXONOMY

Chestnut (*Castanea*) is in the family Fagaceae which also includes oak (*Quercus*) and beech (*Fagus*). There are 13 chestnut species (Camus 1929) native to the temperate zones of Asia, Southern Europe, and the eastern United States. The U.S. species, other than the American chestnut, are called chinkapins. They are an imprecisely defined group of four to six species of shrubs and small trees found in the southeast; the Allegheny chinkapin (*C. pumila* Mill.) and the Ozark chinkapin (*C. ozarkensis* Ashe) are the two most common. Taxonomic keys to the species were published by Graves (1961).

Chestnut probably originated in the Orient.

The western extension (perhaps by man) of the genus gave rise in Europe to the European chestnut (*C. sativa* Mill.) and the eastward migration gave rise to several species in North America. The Asian species have evolved in the presence of the blight fungus and hence possess field resistance to the disease. If the progenitor(s) of the American chestnut were ever resistant, they lost this characteristic as they evolved on this continent in the absence of and without the selective pressure of the blight fungus. The highly susceptible European chestnut may have evolved similarly.

## SEXUAL REPRODUCTION

### Flowering and Reproductive Development

Chestnuts are monoecious and the flowers are borne on the current year's growth. Two types of inflorescence are found: the unisexual male catkins, located on the lower parts of the shoot, and the bisexual catkins toward the terminal end of the shoots. Staminate flowers are spirally arranged along the axis of the catkin in clusters of from three to seven. Pistillate inflorescences appear singly or in clusters of two or three at the base of the bisexual catkins. Three pistillate flowers surrounded by the many-bracted involucre are normally found in the true chestnut section of the genus, whereas the chinkapins, e.g. *C. pumila*, have only a single pistillate flower in each involucre. There are seven to nine styles (Nienstaedt 1956).

Flowering occurs after the first leaves have fully expanded and varies somewhat according to species, clone, and season. Most trees flower in June in Connecticut, and the nuts mature in the fall of the same year. The lower, unisexual male catkins are the first to start opening. The pistillate flowers are next to open and not until 8 to 10 days after anthesis of the unisexual catkins do the male flowers of the bisexual catkins begin to open. Stout (1928) described this sequence of male, female, then male maturation of flowers as *duodichogamy*.

### Self- and Cross-Fertilization

Self-fertilization is rare in chestnuts. This is not due to the differential maturation of the flowers, because there is ample overlap in the flowering periods of the male and female flowers. The cause is self-incompatibility (Clapper 1954, Nienstaedt 1956).

There is disagreement as to whether chestnuts are predominantly wind- or insect-pollinated. Groom (1909) stated that male catkins are predominantly designed for insect pollination: they are long, conspicuous, and grouped; they are scented and nectar is present; pollen grains cling together; and flying insects visit the flowers. However, the female flowers are best suited for wind pollination: the flower is inconspicuous, devoid of odor, and the styles are long, similar to those of wind-pollinated species. Groom concluded that the chestnut is in a transitional stage, changing from an insect-borne to a wind-borne pollinating mechanism. Clapper (1954) found that wind-borne pollen effected pollination through an insect-proof screen and he, as well as Breviglieri (1955), demonstrated that windborne pollen could be trapped on greased slides at a distance of at least 200 feet from the trees. They both concluded that wind is the more important factor in pollination, insects not being necessary.

## Controlled Pollination

Female flowers are not receptive until 5 days after anthesis and remain receptive up to the 17th day (Clapper 1954, Nienstaedt 1956). Best results with controlled crosses can be expected if the pollinations are made between the 10th and 13th day after anthesis. Prior to isolation, the staminate catkins are removed and the male bearing portion of the bisexual catkins pinched off. This eliminates the possibility of self-pollination. Emasculation does not injure the female flowers and may even have a beneficial effect, while bagging does cause some reduction in nut set.

Isolation of female flowers is provided by a paper bag enclosing the entire branch tip. The neck of the bag is usually fastened securely to the past year's growth to prevent breakage of the new growth. Closure is with a piece of wire drawn tightly and given a single twist so it can be readily removed. Male flowers are bagged in a similar manner. When the female flowers are receptive, the bag on the pistillate flowers is removed just long enough to perform the cross pollination. A male catkin shedding pollen is removed from a bag on the tree that is to serve as the male parent and drawn across the stigmas of the female flowers. The bags are then replaced. When the danger of outcrossing has passed (2-3 weeks), the paper bags are removed from the developing burrs and replaced with coarse-mesh cloth bags. These bags serve to mark the location of pollinations, prevent the loss of identifying tags, and retain the hybrid nuts when they mature.

Dry pollen can be stored at low temperatures. Catkins, previously bagged and dehiscing pollen, are dropped onto waxed paper and the pollen is scraped off onto a small stick inserted in a cork which is then fitted into a glass vial. Room is left in the bottom of the vial below the stick for a small amount of desiccant (silica gel). After 4 to 8 hours of drying, the vial containing pollen is placed in storage at  $-15^{\circ}\text{C}$ . Chestnut pollen has retained viability when stored for a year in this manner (Jaynes 1964). It is a valuable technique when the flowering times of parent trees are widely separated or the parents are distant from each other.

Pollen viability can be checked *in vitro*

after incubation at warm temperatures (Nienstaedt 1956). Jaynes (1961b) obtained good germination after incubation for 1 hour at  $30^{\circ}\text{C}$ . in 0.5 percent aqueous sucrose solution.

## Seed Handling

Fresh chestnuts contain 40 to 45 percent percent carbohydrates, mostly in the form of starch, about 5 percent oil, and 50 percent moisture. They are highly perishable because they lose water rapidly under normal humidity conditions, causing the kernel to harden and become ungerminable. Stratification for 1 to 3 months at  $0-4^{\circ}\text{C}$ . is required to break embryo dormancy and insure uniform germination.

As soon as a few burrs on a tree begin to crack open and some of the enclosed nuts begin to turn from a pale green to brown color, the burrs are knocked off or picked, using stout leather gloves to protect the hands from the spiny burrs. This insures harvest of nuts at one time and minimizes losses to animals. The alternative is daily harvesting of nuts from the ground for a period of about 2 weeks.

The burrs are stored in wire crates in a cool ( $18^{\circ}\text{C}$ .), humid room, such as a dirt cellar. The nuts continue to mature and within 5 to 10 days the burrs open. Nuts are separated from the debris and placed in plastic bags along with some nearly dry peat moss for cold storage ( $1-2^{\circ}\text{C}$ .). A slight loss of moisture (curing) is desirable before storing. As long as free water from condensation does not develop in the bags, growth of mold organisms is limited. Normal storage is from fall harvest until spring planting time. However, nuts can be maintained in viable condition for up to  $3\frac{1}{2}$  years, and seed storage for 18 months or until the second planting season after harvest appears to be practical (Jaynes 1969a).

Nuts should be sown in a light, well drained soil with a pH of about 5.5. Fall planting is satisfactory if rodents, especially squirrels, are not a threat. Nuts are covered with 1 or 2 inches of soil and, before the ground freezes, mulched with 2 inches of straw or similar material to prevent damage from low winter temperatures. For spring planting, nuts are stored as described and planted when the soil

can be worked. Seedlings should be allowed 36 square inches of space for the first year's growth. Under good conditions, 1-year seedlings should attain a height of at least 1 foot and a basal diameter of  $\frac{1}{4}$  inch. As with walnuts, the shoot and root of a nut will expend less energy and be straighter if

the nut is planted on its side instead of having the embryo pointing directly down or up. The use of residual (Princep) and knockdown (paraquat) herbicides has proved valuable in controlling weeds in nurseries and young plantations (Ahrens 1969).

## ASEXUAL REPRODUCTION

### Rooting

Vegetative propagation of chestnut is, at best, laborious; and no technique has yet proved to be practical on a large commercial scale. Rooting of greenwood and hardwood cuttings has been attempted in many ways by many people but results have been discouraging (Cummins 1970). As with many other plants, young seedlings or suckers from older plants root most readily. Work by Vieitez et al. (1966) showed that inhibitors present in dormant cuttings taken from old plants could be leached out in running water over a 5-month period and the cuttings rooted. Perhaps a more rapid means to obtain the same effect can be worked out. At East Malling, apple and quince cuttings have been rooted in late winter (1969). Long scions (18 inches) from hedged plants treated with indolebutyric acid were placed in a medium so that the scion base was maintained at 21°C and the tops kept cool, 4–10°C. Perhaps a similar technique can be adapted for chestnut. In Europe, marcottage—or stooling—of selected clones has been practiced successfully but the technique is laborious (Solignat 1964). The rooting of scions by a buried-inarch graft has also met with limited success (Jaynes 1961a).

A promising method of rooting cuttings was described by Shreve and Miles in 1972. Softwood cuttings were taken from current season's shoots derived from the juvenile zone of the tree or adventitious buds. The cuttings were dipped for 1 second in a solution of 0.8 percent indolebutyric acid in ethyl alcohol, and rooted under intermittent mist. I have confirmed (unpublished) the suc-

cess of this method with several chestnut clones.

### Grafting

Various forms of grafting have been widely tested with somewhat inconsistent results. Incompatibility of grafts between, as well as within, species is known. Such problems are reduced if selected clones are grafted on their own seedlings (McKay and Jaynes 1969). Cold injury during the first winter is often a problem but, based on work with pecan (Guengerich et al. 1967), this can be lessened and more growth obtained if the scion wood is "juvenile", that is, taken from basal sprouts or plants which were hedged and not allowed to mature.

One of the easiest and most effective grafting techniques is the simple splice graft (Nienstaedt and Graves 1955). Early in the growing season, but after the danger of frost, 1-year-old seedlings are bench grafted near the root collar using dormant scions of approximately the same diameter. The scion and stock are cut on a slant such that the cut surface is at least four times as long as the diameter. After wrapping and waxing, the grafts are set out in the nursery. Splice grafts can also be made in the field on 2-year-old seedlings with the graft placed at the point on the stock where the scion and stock diameter are approximately the same.

Nut grafting, the grafting of a dormant scion into a germinating nut, has offered the means to graft without the necessity of growing stock plants (Jaynes and Messner 1967). Unfortunately, consistently good results have not been obtained.

# THE CHESTNUT BLIGHT FUNGUS AND HOST RESISTANCE

The chestnut blight fungus is a wound parasite which attacks the phloem of susceptible trees, generally forming a girdling canker on the trunks. The pathogen is an Ascomycete which can be readily cultured on sterile media. Conidia are produced under such conditions, but perithecia and ascospores, products of sexual reproduction, have not yet been obtained in culture. However, in recent studies (Puhalla and Anagnostakis 1971) on the genetics and nutritional requirements of the blight fungus, perithecia and ascospores were formed in the laboratory on chestnut stem segments inoculated with genetically marked strains. Ascospore analysis suggested that the fungus is homothallic but will preferentially outcross. Heterocaryons and putative diploids were formed.

Two toxins, diaporthin and skyrin, produced by the fungus have been implicated as contributing to the disease but it has not been shown that they are essential to disease development. Neither toxin has apparently been isolated from diseased tissue (Owens 1969).

Stands of the European chestnut in France and Italy have suffered severely from the chestnut blight, but its spread has been slower than that witnessed among stands of the American chestnut (Biraghi 1966) even though European chestnut trees grown in the United States are as susceptible to the blight fungus as the American chestnut.

Perhaps the slower spread of the disease in southern Europe is related to climatic factors, such as a lower mean relative humidity, which might inhibit spore germination. The possible role in chestnut stands of a hypovirulent form of the fungus discovered in France by Grente (1965) is not known at this time. The Chinese and Japanese chestnuts are the only two species grown in the United States with notable field resistance to the canker fungus.

The basis for host resistance is not known although Nienstaedt (1953) suggested that relative resistance among species was, at least in part, a result of the differential solubility and qualitative differences among the tannins. This work was not confirmed by Bazzigher (1957a).

Resistance may prove to be a combination of mechanical and chemical factors. Wound periderm does form in advance of the fungal hyphae (Bramble 1936), but the cork and callus tissues are seldom able to permanently stop the host pathogen (Bazzigher 1957b). Resistance is probably not passive, but active, and apparent only after invasion of host tissue.

The chestnut blight fungus is a threat to certain other trees, especially live oak (Battson and Witcher 1968, Stipes and Phipps 1971). However, barring a change in virulence of the fungus, there is little likelihood that damage on live oak will ever approach that caused to the American chestnut.

## GENETICS AND BREEDING

### Species Variability and Chromosome Numbers

The thirteen species of chestnut are individually and collectively highly variable. Nut weight ranges from 1,000 to 10 nuts per pound (2,200 to 22 nuts per kilogram), onset of flowering varies over a period of 6 weeks, fruit maturity varies from early September to late October in Connecticut, growth habit varies from small shrubs to tall trees, and resistance to the blight fungus ranges from the near immune to the highly susceptible.

In fact, virtually all traits of chestnut show wide variation. Recombination of selected traits into trees of superior genotype is a challenge to the geneticist and plant breeder.

Provenance studies have not been conducted with any of the chestnut species, but surely they would demonstrate a wide range of variation for morphological and physiological traits. Such a study of the Chinese, Japanese, or European chestnut would be confounded by the inability to separate wild or native trees from cultivated or escaped trees.

In the United States, selections of cultivated forms of the American chestnut were just beginning to be developed when the bark disease was introduced (Heiges 1896).

The normal somatic chromosome number for chestnut is 24. Ten species and several interspecific hybrids were examined (Jaynes 1962) and all had  $2n=24$  except for two hybrids: one was a triploid and the other had  $2n=25$ . A technique for successfully doubling the chromosomes of chestnut was described by Dermen and Diller (1962) and Genys (1963).

### Field Resistance of American Chestnut

Resistant American chestnut trees have been sought since shortly after the blight fungus was discovered. Diller (1957), personally and through cooperators, kept data on several hundred large surviving trees for years. MacDonald and Thor (1967) classified over 200 clones in the Southern Appalachian Mountains where the majority of the trees had a dbh of 15 to 23 inches. The existence of large infected trees within the native range is evidence for different levels of resistance within the American chestnut. Unfortunately, none of these surviving natives have the combination of characters desired, namely, good form, vigorous growth, and a high degree of resistance. They are generally derelicts. MacDonald et al. (1962) and many others have made efforts to intercross the most resistant American chestnuts to recover plants with field resistance. The Stronghold Corporation (1972), with similar aims, has recently publicized its desire to learn of large surviving American trees. To date, however, evidence for a high degree of resistance within the American chestnut is lacking.

There are scattered large, uninfected American trees in the the midwestern states, such as Illinois, Michigan, and Wisconsin, and in the West in California, Oregon, and Washington. There are also uninfected escapes on the fringes or just outside the native range (fig. 2) of the American chestnut. These trees are generally not exposed to the spores because of the prevailing westerly winds and the lack of suitable host plants to maintain the fungus. However, within the range in the East the numerous recurring diseased American chestnut sprouts provide a continual supply of inoculum.

MacDonald et al. (1962) and Singleton and Dietz<sup>2</sup> have irradiated American chestnut seed to induce mutations for increased disease resistance. The LD 50 has not been determined exactly, but for nuts treated in the spring just prior to germination, Thor and Barnett<sup>3</sup> determined the LD 50 as 6,000r. Evaluation and utilization of the resistance that may occur naturally or that which may be induced by irradiation in the American chestnut is still in the early stages of development.

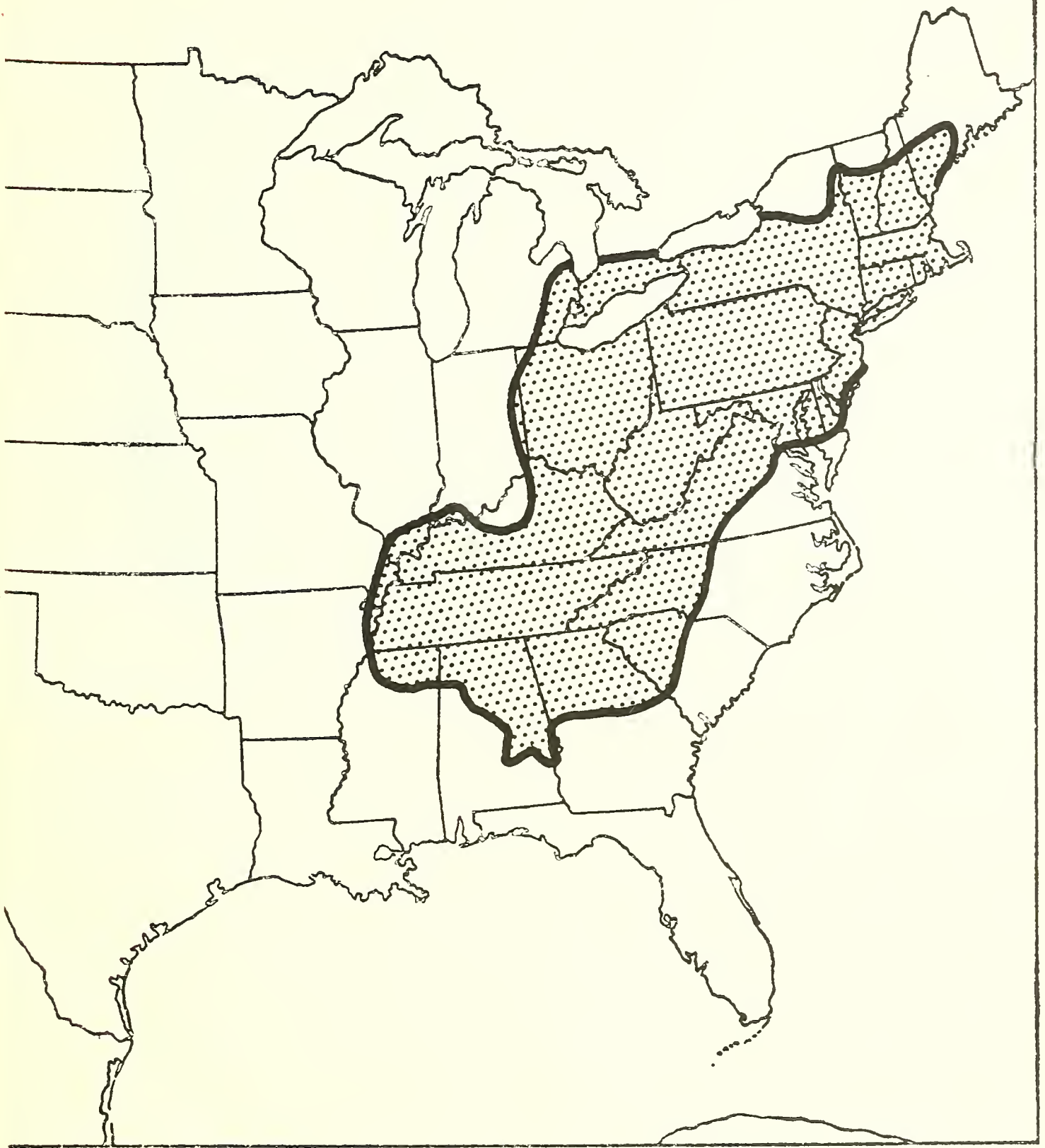
### Interspecific Hybridization

Chestnut breeding began in the United States before the chestnut blight fungus was discovered. About 1890, George W. Endicott crossed the Japanese and the American chestnut (Crane et al. 1937, Detlefsen and Ruth 1922), and at about the same time Luther Burbank (1921) embarked on a chestnut hybridizing program. Two institutional programs have included most of the forest tree breeding: one, the USDA project located at and near Beltsville was begun in 1894 and carried on until 1954. The second was initiated at the Brooklyn Botanic Garden in 1929 and later continued at the Connecticut Agricultural Experiment Station to the present. Much of the chestnut breeding has had the goal of developing a forest tree with form and growth characteristics similar to the American chestnut plus resistance to the blight fungus as obtained from the Chinese or Japanese chestnut (fig. 3).

Hybridization among species has not been difficult; in fact most of the possible  $F_1$  crosses have been attempted and completed successfully (Fig. 4). There is little evidence of incompatibility, except for the occasional presence of male sterility among  $F_1$  and later generations (Jaynes 1964). Inheritance of catkin length, indeterminant flowering, male sterility, and nut flavor, as well as influence of the male parent on nut size, dormancy, and kernel color are reported or reviewed by Clapper (1954), Jaynes (1961b, 1963), and McKay (1960).

<sup>2</sup> Personal communication with Singleton.

<sup>3</sup> Personal communication with Thor.



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FIGURE 2. Range of the American chestnut (after Harlow and Harrar 1958).



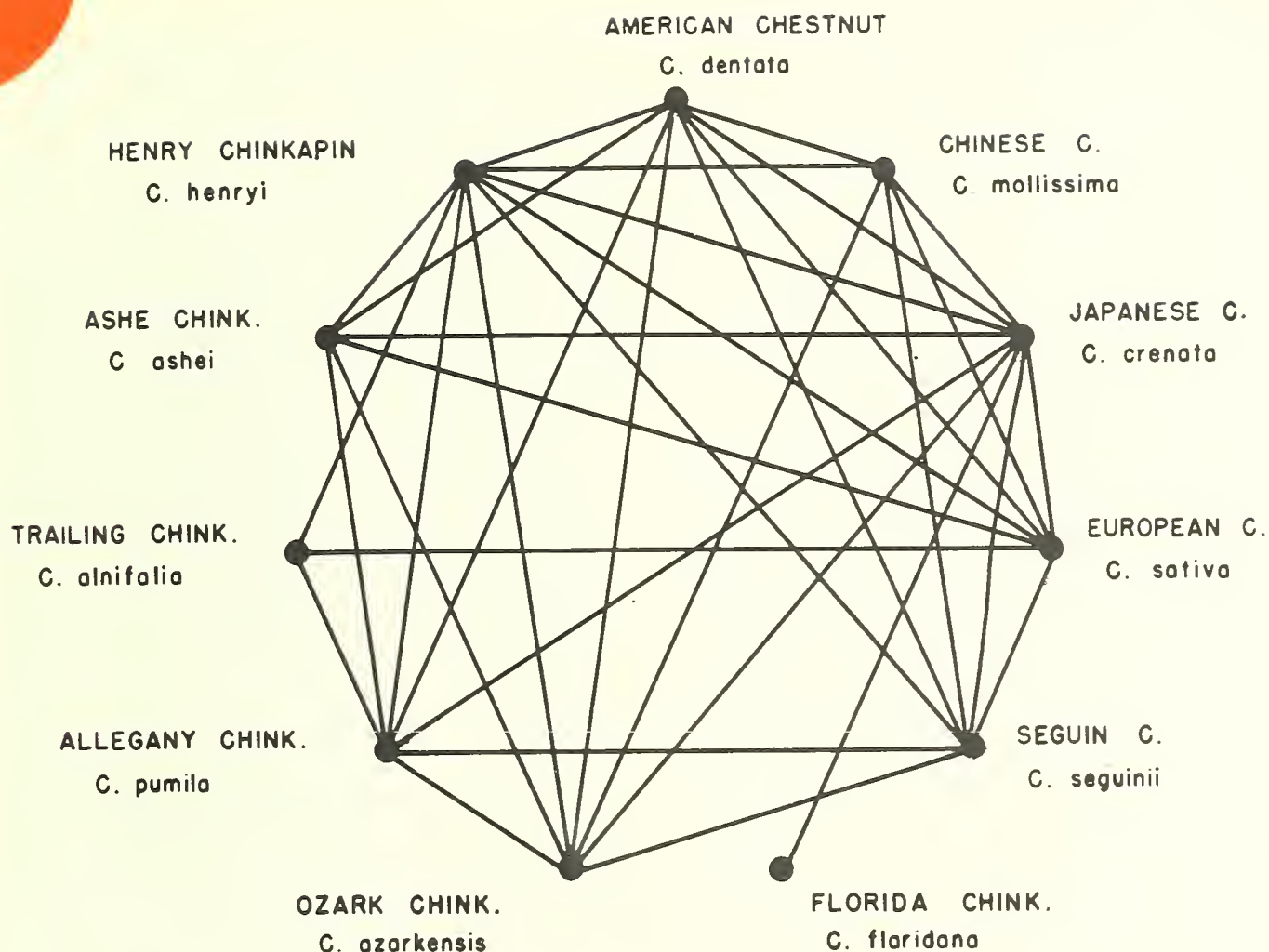
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Figure 3. Leaves and twigs of three chestnut species. *Left*, Chinese chestnut—winter twig color is light, leaf is broad, and angle of base of leaf blade to petiole approaches  $180^\circ$ . *Center*, Japanese chestnut—buds are rounded, twig color is purplish brown, leaf is narrow, margin is bristle tipped, and base of leaf is blunt. *Right*, American chestnut—buds pointed, dentate leaf margin, basal angle of leaf blade is acute. Size of leaves varies and is not critical in identification.

The inheritance of form and vigor, like blight resistance, is not well known and is probably under polygenic control. First generation hybrids of the American chestnut with the Chinese or Japanese are generally vigorous and have many of the favorable attributes of the American chestnut (Clapper 1954, Nienstadt and Graves 1955), but they are intermediate in blight resistance and lack good field resistance. Third and fourth generation hybrids of these three species most often have a spreading habit if resistant

to the blight, and, if columnar in form, they are susceptible (Jaynes and Graves 1963 and personal observation).

As with all forest trees, the screening of young chestnut trees to determine their future performance is desirable but full of problems. The ratio of tree height to crown diameter of 3-year-old seedlings gave an indication of future form in a comparison of species hybrids with American seedlings (Jaynes and Graves 1963), but height alone was a poor measure for predicting vigor.



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Figure 4. Summary of successful controlled crosses between chestnut species as indicated by solid lines (Jaynes 1969b).

### Genetics of Resistance

Clapper (1954) concluded from analysis of Chinese-American  $F_1$  and  $F_2$  hybrids that at least two recessive genes were involved in blight resistance. However, because of the apparent continuous gradation of classes from resistant to susceptible, the inheritance is likely polygenic rather than oligogenic. In the terms of Van der Plank (1968), resistance is probably horizontal rather than vertical. Horizontal resistance is characterized as being stable and usually of a polygenic nature; it is also difficult to handle and evaluate in a breeding program. Vertical resistance is fickle; it is controlled by relatively few genes in the host, and may be overcome

by single gene mutations in the pathogen. In perennials, and trees especially, we should strive for horizontal resistance. This is even more important in view of the evidence that *E. parasitica* is variable (Andes 1961) and strains may be nonpathogenic or hypovirulent (Grente 1965, Puhalla and Anagnostakis 1971).

The Chinese and Japanese chestnut species are not uniformly resistant, a fact often overlooked by those writing about breeding resistant chestnuts. Indeed, blight cankers are common on some trees of these "resistant" species (Diller et al. 1964, Jaynes 1967). Field resistance of hybrids is understandably difficult to achieve, therefore, when resistance factors of these Oriental species are

diluted with genes from a susceptible species such as the American chestnut.

### Selected Blight Resistant Trees

Many test plots of hybrid and Chinese chestnuts have been established over the years. Diller et al. (1964) reported on 15 cooperative plots established between 1947 and 1955 with Chinese chestnuts and species hybrids from the USDA and Connecticut breeding projects. Desirable trees were noted on 11 of the 15 plots. No particular source or hybrid showed consistently high performance on all plots. The best Chinese source was PI 58602. Diller and Clapper (1965, 1969) recommended this strain for timber plantings, but Jaynes (1967) found the form poor on a Connecticut site although extensive natural regeneration had occurred. This Chinese strain was considered excellent as an annual food producer for wildlife.

The most outstanding tree in the cooperative plots was a hybrid in the test planting at the Crab Orchard National Wildlife and Game Refuge. This tree, named "Clapper" for the breeder who produced it, is a complex hybrid of the Chinese and American species (fig. 5) (Clapper 1963). In 1971 it had excellent form and at the age of 24 years was 66 feet tall and 12.5 inches dbh.

This remarkable tree is a case in point of two obstacles limiting progress in breeding chestnuts, namely vegetative propagation and screening for blight resistance. The Clapper chestnut has neither been propagated for test in other plantings nor has it been adequately tested for resistance to the chestnut blight fungus. Similarly, six other hybrids named by Graves (1960, 1962) have not been adequately tested.

Nichols et al. (1971) evaluated the 24-year-old chestnut plot at Table Rock, S.C., which was one of the 15 established by Diller. On the basis of the performance of the best trees in this planting, as well as others, they concluded that the prospects of tree breeders bringing the chestnut back are brighter now than ever before.



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FIGURE 5. Clapper hybrid chestnut after 25 growing seasons [(*C. mollissima* x *C. dentata*) *C. dentata*]. A natural infection of the chestnut blight appeared at the base of the tree several years ago. A serious canker has not developed to date.

## IMPROVEMENT PROGRAMS

### Screening for Disease Resistance

A reliable test is needed to determine field resistance of trees to the chestnut blight fungus without having to wait 5 to 25 years for natural infection to occur. Young trees are susceptible to the disease and can be inoculated. Unfortunately, growth of the fungus from artificial inoculations is not closely correlated with known field resistance of the tree. While such a test is not crucial for older selections which have already withstood natural infection, the lack of a satisfactory test is a great handicap in developing new and better selections. Similarly, this lack has limited the study of the genetic control of resistance.

A screening method is needed for 1- or 2-year-old trees that will allow all seedlings to be culled which would later prove to have inadequate field resistance. If an efficient method were devised to test seedlings in a nursery, thousands of young plants could be screened and only the resistant ones kept for field planting and further evaluation of form, vigor, nut quality, etc.

The existence of occasional large American chestnut trees further illustrates the problem of evaluating blight resistance. There is one such tree in Amherst County, Va. (well within the native range). The tree trunk is about 3 feet in diameter and the bark is and has been infected by the canker disease for several years. Yet the tree is alive. At the base there are some young sprouts and these too have become infected and are being killed by the disease. It is unlikely that the sprouts are genetically different from the main stem. J. G. Horsfall<sup>4</sup> believes that such trees have partial resistance by virtue of their not setting fruit. Fruit fails to set because there are no other flowering chestnuts nearby to effect cross-fertilization. The increased resistance of the non-fruiting plant may be similar to the situation in vegetable crops such as tomatoes, where susceptibility to pathogens is correlated with fruiting and the

subsequent decrease in carbohydrates in the plant sap (Horsfall and Dimond 1957). Yet other large trees such as those selected by the University of Tennessee and the Virginia Division of Forestry do fruit, are infected, but remain alive.

### Systemic Fungicides

The chemotherapeutic value of fungicides in controlling the chestnut blight fungus has received little attention since 1920 (Rumbold). However, in 1971 Jaynes and Anagnostakis showed that benomyl applied as a soil drench on 2-year-old American chestnut trees significantly limited growth of *E. parasitica*. This was the first report of a fungicide with fungistatic effect on the chestnut blight fungus in the field. Other systematic fungicides should be tested. Although such agents may prove too costly to use on a forest-tree planting, their value on test plantings and ornamentals could be significant.

### Research Needs

Recent efforts in breeding and selecting hybrid chestnut trees have concentrated on modified seed orchard technique (Jaynes 1969b). Open pollinated seed is collected from the best trees in small plantings where there is assurance that the pollen source is also confined largely to the selected trees. In this manner the laborious process of controlled pollinating is avoided and more seed is obtained, yet genetic gain per generation may be as rapid as from controlled crosses. Since chestnuts are essentially self-sterile, selfing is not a problem. The ultimate objective is the production of a blight resistant timber-tree, but of immediate value are selections for nut orchards, ornamentals, and sources of food for wildlife.

It is possible that, in time, essentially true breeding selections could be developed, eliminating the need for vegetative propagation. Pairs of clones are needed that will intercross when grown in isolated plantings

<sup>4</sup> Personal communication.

(seed orchards) and produce a high percentage of offspring as good as the two parent selections.

Fourth generation hybrids among the Chinese, Japanese, and American chestnut are now being grown. In a cooperative effort involving the Virginia Division of Forestry, the Connecticut Agricultural Experiment Station, the Lesesne Foundation, and the Accokeek Foundation, several thousand hybrid seedlings have been established in Virginia. Progress in breeding blight-resistant chestnuts has been slow, but improved trees have been developed and each generation of hybrids should continue to reveal better clones and lines.

Research is needed on the nature of host resistance, amount of resistance in the American chestnut, natural variation of pathogen virulence, rapid screening of seedlings for disease resistance, the potential of radiation breeding, and improved means to vegetatively propagate chestnut. Much remains to be done, but the recent efforts of scientists and foresters at Clemson College, the Connecticut Agricultural Experiment Station, the University of Tennessee, the Virginia Division of Forestry, and elsewhere give hope to continued progress in the development of blight resistant chestnut trees.

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